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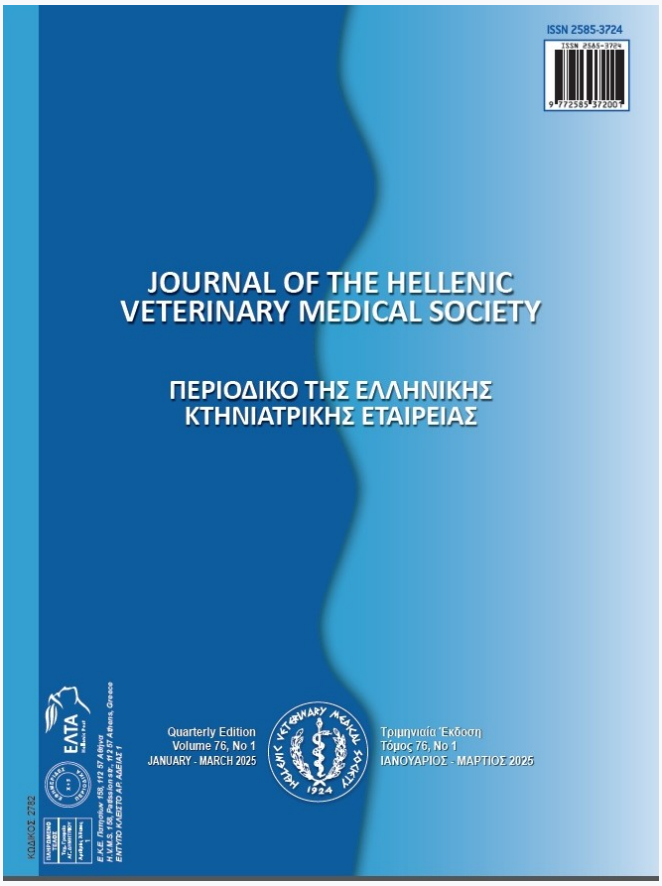

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Research article
Ερευνητικό άρθρο

Prevalence and risk factors of viral diseases in broiler farms contracted to multi-factorial respiratory syndrome

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ABSTRACT: *Newcastle Disease (ND)*, *Avian Influenza (AI) H9N2* and *Infectious Bronchitis (IB)* are the major respiratory diseases affecting broiler poultry farming in Iran. This study aims to investigate the prevalence and risk factors associated with these viral diseases in commercial broiler chicken farms experiencing multi-factorial respiratory syndrome in the country. In this cross-sectional study, 157 affected commercial broiler chicken farms were sampled from 2017 to 2019. Samples were examined for *AIV H9N2*, *NDV*, and *IBV* by performing reverse transcription polymerase chain reaction. Possible risk factors for the occurrence of viral diseases were investigated by comparing the mean and frequency of the factors, as well as performing univariable and multivariable regression analysis. Highest frequency of investigated viral diseases was due to *ND* with 98 cases (62.43%, CI 95%= 54.35-70.01). Frequency of *AI H9N2* and *IB* were respectively 74 cases (47.13%, CI 95%= 39.13-55.25) and 22 cases (14.1%, CI 95%= 8.99-20.44). Flock age (Odd Ratio: 2.65, P value: 0.030) and first week mortality (Odd Ratio: 10.12, P value: 0.014) respectively had significant effect on occurrence of *AI H9N2* and co-infection of *AI H9N2*. In conclusion, it is essential to implement control strategies measures to mitigate the impact of multi-factorial respiratory syndrome in Iranian commercial broiler chicken farms. This should specially focus on prioritize viral diseases based on their prevalence and associated risk factors, determine the serotypes and genotypes of the circulating strains of the virus, and improve biosecurity measures.

Keywords: *Newcastle Disease*; *Infectious Bronchitis*; *Avian Influenza H9N2*, Broiler, Epidemiology

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INTRODUCTION

In recent years, Iran's poultry farming, particularly broiler ones, has experienced significant growth in the country. This rapid expansion and development has led to an increase in the density of these farms in certain regions of the country. Poultry farming in a dense manner can lead to development of complex and multi-factorial infections caused by viruses and bacteria. (Ahmadi et al. 2014).

In Iran's broiler poultry farming, the occurrence of poultry respiratory diseases due to multiple agents called poultry multi-factorial respiratory syndrome, accounts for the highest number of mortality in these farms every year. Multi-factorial respiratory syndrome occurs either as individual infection due to viral agents or simultaneous infection due to presence of several viral and bacterial agents (Saif et al. 2020). Evidence shows that most of these agents have a synergistic effect on each other. Indeed, different mortality rates are observed depending on which of the pathogenic agents are present in the farm. Despite the use of various preventive and control measures as well as widespread use of numerous vaccines, the prevalence and occurrence of respiratory diseases in poultry broiler farms have remained significant in recent years. According to the statistics available from the Iran Veterinary Organization (IVO), the most significant economic losses in the country's broiler farms are associated with the occurrence of multi-factorial respiratory syndrome.

Among the causes of multi-factorial respiratory syndrome, *ND*, *IB* and, *AI H9N2* are endemic in Iran. Their occurrence results in significant losses to broiler farms every year. In recent years, despite national and domestic prevention and control measures against the three diseases, coupled with the widespread use of diverse vaccines, outbreaks of these viral diseases still lead to the appearance of respiratory syndromes in broiler flocks, resulting in high annual mortality (Nili and Asasi 2002, Fallah Mehrabadi et al. 2019a, Hosseini et al. 2015).

Considering the important role of *ND*, *IB* and *AI H9N2* in the occurrence of multi-factorial respiratory syndrome in Iran, it is important to prioritize them based on their prevalence and related factors to design and implement better preventive plans. This study determined the prevalence and risk factors for these diseases in commercial broiler chicken farms which contracted to multi-factorial respiratory syndrome in Iran from 2017 to 2019.

MATERIALS AND METHODS

Study Design and setting

In this cross-sectional study, conducted during 2017 to 2019, 157 commercial broiler chicken farms contracted to multi-factorial respiratory syndrome in Iran were sampled and evaluated.

According to the latest statistics of the Ministry of Jihad Agriculture of Iran, approximately 20,000 commercial broiler chicken farms have licenses in Iran (Ebadzadeh et al. 2015). All of these farms scattered in 31 provinces of the country with different densities. The largest numbers of farms are located in Mazandaran and Isfahan provinces. Likewise, the number of hatching times in a year also varies in different regions, so that it is up to 5 times a year in the Northern provinces of the country and up to 3 times a year in the Southern provinces of the country.

All broiler farms in Iran are under the supervision of a private vet as a farm vet or clinician, in groups of one to several, based on farm population and location. The farmers and their Vet supervisors must give online prompt report when a farm is involved in serious problems, including important diseases as defined by IVO as a National Assignment. So the study population including infected farms was self-reported by farmers or their private Vets supervisor, in addition to farms that were reported following the active surveillance programs of IVO by state Vets

Therefore the studied farm selected among farms contracted to multi-factorial respiratory syndrome reported by IVO during 2017 to 2019 in Iran. The number of required sample in each province was determined based on the proportion of active broiler farms in each province. Farms with multi-factorial respiratory syndrome defined as existence of respiratory symptoms including respiratory rales, chafing, and runny nose, with or without gastrointestinal symptoms including diarrhea and neurological symptoms (wristed neck, paralyzed wings or legs) along with increased losses during at least 3 days.

Data collection

Detection of Viral Agents

For this purpose, initially, 5-7 recently deceased broiler chickens or those with clinical symptoms were sampled in each selected infected farms. The samples included tracheal and cloacal swabs, tracheal tissue, lung, brain, spleen, tonsils and kidney. All collected samples were transported to the diagnostic center lab-

oratory of the Iran Veterinary Organization and the department of poultry diseases at the *Razi vaccine and serum research Institute*. The samples were labeled with their respective characteristics. Furthermore, the samples were stored in a freezer at -70 degrees Celsius until the tests were conducted.

Finally, RT-PCR method was used in order to detect *AIV H9N2*, *IBV* and *NDV* respectively. RNA extraction was done by commercial kits as follow: RNA was isolated from tissue samples using the High Pure RNA Extraction Kit (Roche, Mannheim, Germany). The isolated RNA was subsequently converted into cDNA with the RevertAid™ First Strand cDNA Synthesis Kit (Thermo Scientific, MA, USA), utilizing random primers. The PCR reaction was performed in a 25 µl reaction volume, consisting of 8.5 µl of nuclease-free water, 12.5 µl of Taq DNA Polymerase Master Mix RED (AMPLIQON, Odense M, Denmark), 1 µl of each primer (10 µM) (Table 1), and 2 µl of cDNA. The thermal cycling conditions were as follows: an initial denaturation at 95°C for 2 minutes, followed by 35 cycles of 95°C for 20 seconds, 52-54°C for 30 seconds, and 72°C for 1 minute, with a final extension step at 72°C for 5 minutes. The PCR products were then separated on a 1% agarose gel and visualized under UV light using a gel documentation system. (Monne et al. 2008, Callison et al. 2006).

Possible Risk Factors

For this purpose, a questionnaire was designed that includes general information about the farm and information related to the occurrence of the disease. This questionnaire was completed during sampling and farms visits through observation and face-to-face interview. It should be noted that the relevant questionnaire only was completed in 2017 and 2018. Because of change in the policies of the Iran Veterinary Organization (IVO) in 2019, the data due to questionnaire wasn't collected.

Statistical Analysis

In order to analyze the data, the frequency and relative frequency with 95% CI were used to describe the occurrence of viral diseases.

To assess the factor related to occurrence of the disease (all combinations), we compared the mean, frequency and total indices of the investigated factors. For quantitative variables, t-test and Mann-Whitney test were used and for qualitative variables, chi square test was used.

To assess the factor related to occurrence of the disease (Simple Infection/No Infection) (Co-Infection/Simple Infection), we used univariable and multivariable binary logistic regression analysis. Factor with P value ≤0.1 in univariable regression analysis inserted to multivariable analysis. Eventually in multivariable regression analysis factor with P value ≤0.05 were considered as significant. All Statistical analysis was done using Stata software version 14.

RESULTS

Frequency of Viral Diseases

In this study, samples were collected from 157 active commercial broiler chicken farms, comprising 55, 77 and, 25 farms in 2017, 2018 and, 2019 respectively. In this study, results indicated that the highest frequency of viral disease was due to *ND* with 98 cases (62.43%, CI 95%= 54.35-70.01). Frequency of *AI H9N2* and *IB* were respectively 74 cases (47.13%, CI 95%= 39.13-55.25) and 22 cases (14.1%, CI 95%= 8.99-20.44). (Table 2)

Factor Related to Frequency of Viral Diseases (All Combinations)

Analysis of the data in order to investigate factor related to occurrence of the viral diseases (all combinations), indicated following results:

Table 1: List of primers used in this study

Name	Target gene	Sequence	Amplicon length	Detected of	Reference
MB-NDV-F MB-NDV-R	Fusion	5′-GGTGAGTCTATCCGGARGATACAAG-3′ 5′-TCATTGGTTGCRGCAATGCTCT-3′	202	NDV	(Creelan 2002)
MB-N103F	Nucleocapsid	5′-CCTGATGGTAATTTCCGTTGGG-3′	357	IBV	(Loa 2006)
MB-N102R		5′-ACGCCCATCCTTAATACCTTCCTC-3′			
MB-H9-151f	Hemagglutinin	5′-CTYCACACAGARCACAATGG-3′	488	AIV subtype H9	(Lee et al. 2001)
MB-H9-638r		5′-GTCACACTTGTTGTTGTRTC-3′			
MB-NA2-1	Neuraminidase	5′-TCCGTTTCATTTGGGAACC-3′	314	AIV subtype N2	(Qiu et al. 2009)
MB-NA2-2		5′-CTGACAATGGRCTAATGTG-3′			

There was no statistically significant relationship between the vaccination administration status and the frequency of viral diseases (P>0.05). There was a significant relationship between the flock age and the frequency of viral diseases (P=0.027). Indeed, mean of flock age at syndrome occurrence onset time was higher in farms infected only with *AI H9N2* than other farms. Abundance of *ND* vaccination also had no significant relationship with the frequency of viral diseases (P>0.05). Abundance of *IB* vaccination had significant relationship with the frequency of viral diseases (P=0.001). Indeed abundance of *IB* vaccination in farms infected to *IB* was lower than farms not infected to the disease. First week mortality, had significant relationship with the frequency of viral diseases (P=0.001). Indeed mean of first week mortality was higher in farms only infected to *IB* than other farms. Abundance of imported chick, had no significant relationship with the frequency of viral diseases (P>0.05). Flock age at *AI H9N2* vaccination administration time and *ND* vaccination administration time had no significant relationship with the frequency of viral diseases (P>0.05). (Table 3)

Factor Related to Frequency of Viral Diseases (Simple infection/No Infection)

Analysis of the data in order to investigate factor related to occurrence of the viral diseases (simple infection/no infection), indicated following results:

Investigation regarding relationship of the factor and frequency of *AI H9N2* indicated that age flock has significant effect on *AI H9N2* in the univariable and multivariable regression analysis. Indeed, in multivariable regression analysis we indicated that farms with age flock 30-60 days had the higher odds of *AI H9N2* related to the farms with age flock less than 30 days (Odd Ratio: 2.65, P value: 0.030). There was

Table 2: Frequency and Relative Frequency of Viral Disease in Commercial Broiler Chicken Farms contracted to Multi-Factorial Respiratory syndrome in 2017 - 2019

Viral Disease	2017		2018		2019		Total	
	Frequency	Relative Frequency	Frequency	Relative Frequency	Frequency	Relative Frequency	Frequency	Relative Frequency
AI H9N2	26	47.3	16	20.8	5	20	47	29.94
ND	20	36.4	43	55.8	5	20	68	43.31
IB	1	1.8	2	2.6	2	8	5	3.18
AI H9N2 + IB	3	5.5	0	0	4	16	7	4.46
ND + AI H9N2	4	7.3	13	16.9	3	12	20	12.74
ND + IB	1	1.8	3	3.9	6	24	10	6.37
Total	55	100	77	100	25	100	157	100

Table 3: Factor Related to Occurrence of Viral Diseases (all combinations) in Commercial Broiler Chicken Farms contracted to Multi-Factorial Respiratory Syndrome in 2017 - 2018

Independent Factor		*AI H9N2*	*ND*	*IB*	*AI H9N2 + IB*	*ND + AI H9N2*	*ND + IB*	P value
Flock Age at Syndrome Occurrence Onset Time (Day)	Mean	34.6	29.88	29.33	29.66	32.23	29.25	0.027
	SD	9.60	7.37	6.11	6.42	4.07	9.53	
	Min	13	15	24	25	23	19	
	Max	59	42	36	37	39	42	
	Median	31	31	28	27	32	28	
Abundance of Imported Chicks (Number)	Mean	19479.64	19117.02	18400	30633.33	27153.53	14609.25	0.360
	SD	13051.16	13453.16	9699.485	11623.4	27107.65	6669.488	
	Min	3000	5002	7200	22900	6000	10000	
	Max	74000	93300	24000	44000	121300	24437	
	Median	17750	17900	24000	25000	20500	12000	
First Week Mortality (Number)	Mean	2675.35	7458.31	9095.33	9563	8166.35	4120	0.001
	SD	3994.55	6705.55	4582.73	4153.54	8690.62	3048.47	
	Min	40	220	4050	6626	1600	860	
	Max	19000	38000	13000	12500	39329	6900	
	Median	1200	5340	10236	9563	5250	4600	

Vaccination Administration Status according to Viral Agents	*AI H9N2* Vaccine Administration	Yes, N=72	23	37	3	1	7	1	0.120
		No, N=60	19	26	0	2	10	3	
	IB Vaccine Administration	Yes , N=103	30	50	3	2	15	3	0.490
		No, N=29	12	13	0	1	2	1	
	ND Vaccine Administration	Yes, N=126	40	61	3	2	17	3	0.055
		No, N=6	2	2	0	1	0	1	
	ND Vaccine Administration (Injectable Form)	Yes, N=80	28	39	3	1	7	2	0.100
		No, N=52	14	24	0	2	10	2	
	AI H9N2+ *IB*+ *ND* Vaccine Administration	Yes, N=57	18	29	3	1	6	0	0.110
		No, N=75	24	34	0	2	11	4	
Flock Age at Newcastle Vaccination administration Time (Day)		Mean	5.52381	5.650794	8.666667	4.666667	7.058824	6.25	0.250
		SD	3.81421	3.208651	1.154701	4.163332	1.983387	4.5	
		Min	0	0	8	0	3	0	
		Max	11	11	10	8	11	10	
		Median	5.5	7	8	6	7	7.5	
Flock age at *AI H9N2* Vaccination administration Time (Day)		Mean	4	3.95	8.66	2	2.88	2.5	0.100
		SD	4.29	4.01	1.15	3.46	3.58	5	
		Min	0	0	8	0	0	0	
		Max	11	11	10	6	8	10	
		Median	2	4	8	0	0	0	
Abundance of *ND* Vaccination (Injectable Form)		0	2	2	0	1	0	1	0.260
		1	10	8	0	0	3	1	
		2	7	11	0	1	3	1	
		3	6	22	1	0	6	0	
		4	8	12	2	1	5	1	
		≥5	9	8	0	0	0	0	
		All	42	63	3	3	17	4	
Abundance of *IB* Vaccination		0	13	8	0	2	5	1	0.001
		1	11	40	0	1	6	1	
		≥2	18	15	3	0	6	2	
		All	42	63	3	3	17	4	

no statistically significant relationship between the other possible factor and the frequency of *AI H9N2* diseases. Also there were no statistically significant relationship between the possible factor and the frequency of *ND* diseases. In addition, there were no statistically significant relationship between the possible factor and the frequency of *IB* disease. (Table 4)

Factor Related to Frequency of Viral Diseases (Co-Infection /Simple Infection)

Analysis of the data in order to investigate factor related to occurrence of the viral diseases (Co-Infection/Simple Infection), indicated following results:

Investigation regarding relationship of the factor and frequency of co-infection of *AI H9N2* indicated that first week mortality has significant effect on co-infection of *AI H9N2* in the univariable and multivariable regression analysis. Indeed in multivariable regression analysis we indicated that first week mortality above 8000 can increase odds of occurrence of co-infection *AI H9N2* related to those first week mortality was less than 4000 (Odd Ratio: 10.12, P value: 0.014). There was no statistically significant

relationship between the other possible factor and the frequency of co-infection of *AI H9N2* diseases.

Moreover, there were no statistically significant relationship between the possible factor and the frequency of co-infection of *ND* diseases. As the same, there were no statistically significant relationship between the possible factor and the frequency of co-infection of *IB* diseases. (Table 5)

Table 4: Factor Related to Occurrence of Viral Diseases (Simple Infection/No Infection) in Commercial Broiler Chicken Farms contracted to Multi-Factorial Respiratory Syndrome in 2017 - 2018

Viral Disease	Factor		Univariable Regression Analysis				Multivariable Regression Analysis			
			Regression Coefficient	SD	OR	P-value	Regression Coefficient	SD	OR	P-value
AI H9N2	Flock Age at Syndrome Occurrence Onset Time (Day)	<30	-	-	-	-	-	-	-	-
		30-60	1.31	0.431	3.70	0.002	0.97	0.45	2.65	0.030
		>60	22.48	9748.2	5815709434	0.998	21.956	9669.182		0.998
	Abundance of Imported Chicks (Number)	<10000	-	-	-	-	-	-	-	-
		10000-20000	0.50	0.47	1.66	0.282	-	-	-	-
		>20000	0.58	0.47	1.79	0.214	-	-	-	-
	First Week Mortality (Number)	<4000	-	-	-	-	-	-	-	-
		4000-8000	-1.59	0.47	0.20	0.001	-1.028	0.51	0.35	0.055
		>8000	-1.17	0.46	0.31	0.011	-0.704	0.49	0.49	0.495
	AI H9N2 Vaccine Administration	Yes	-0.346	0.35	0.70	0.324	-	-	-	-
		No	-	-	-	-	-	-	-	-
ND	Flock Age at Syndrome Occurrence Onset Time (Day)	<30	-	-	-	-	-	-	-	-
		30-60	-1.089	0.48	0.337	0.0.24	-0.68	0.50	0.50	0.178
		>60	-22.92	9748.22	0	0.998	-22.342	9628.88	0	0.998
	Abundance of Imported Chicks (Number)	<10000	-	-	-	-	-	-	-	-
		10000-20000	-0.409	0.49	0.66	0.405	-	-	-	-
		>20000	-0.326	0.49	0.72	0.508	-	-	-	-
	First Week Mortality (Number)	<4000	-	-	-	-	-	-	-	-
		4000-8000	1.70	0.51	5.50	0.001	1.12	0.58	3.09	0.053
		>8000	1.59	0.52	4.91	0.002	1.11	0.58	3.04	0.056
	ND Vaccine Administration	Yes	0.588	0.83	1.80	0.483	-	-	-	-
		No	-	-	-	-	-	-	-	-
	ND Vaccine Administration (Injectable Form)	Yes	-0.405	0.377	0.66	0.283	-	-	-	-
		No	-	-	-	-	-	-	-	-
	Abundance of *ND* Vaccination (Injectable Form)	0	-	-	-	-	-	-	-	-
		1	0.182	0.92	1.20	0.843	-	-	-	-
		2	0.629	0.92	1.87	0.497	-	-	-	-
		3	1.38	0.91	4	0.132	-	-	-	-
		4	0.492	0.90	1.63	0.585	-	-	-	-
		$\geq_5$	-0.118	0.95	0.88	0.901	-	-	-	-
IB	Flock Age at Syndrome Occurrence Onset Time (Day)	<30	-	-	-	-	-	-	-	-
		30-60	-1.37	0.71	0.25	0.056	-1.44	0.86	0.23	0.093
		>60	-19.48	9748.22	0	0.998	-18.49	9559.031	0	0.998
	Abundance of Imported Chicks (Number)	<10000	-	-	-	-	-	-	-	-
		10000-20000	-0.56	1.02	0.57	0.586	-	-	-	-
		>20000	0.62	0.85	1.86	0.463	-	-	-	-
	First Week Mortality (Number)	<4000	-	-	-	-	-	-	-	-
		4000-8000	2.079	1.14	8	0.068	1.49	1.16	4.43	0.200
		>8000	1.861	1.17	6.42	0.114	1.46	1.19	4.33	0.220
	IB Vaccine Administration	Yes	-0.459	0.725	0.632	0.526	-	-	-	-
		No	-	-	-	-	-	-	-	-
	Abundance *IB* Vaccination	0	-	-	-	-	-	-	-	-
		1	-1.19	0.943	0.30	0.20	-	-	-	-
		$\geq_2$	0.10	0.773	1.11	0.89	-	-	-	-
		All	-	-	-	-	-	-	-	-

Table 5: Factor Related to Occurrence of Viral Diseases (Co-Infection/Simple infection) in Commercial Broiler Chicken Farms contracted to Multi-Factorial Respiratory Syndrome in 2017 - 2018

Viral Disease	Factor		Univariable Regression Analysis				Multivariable Regression Analysis			
			Regression Coefficient	SD	OR	P-value	Regression Coefficient	SD	OR	P-value
Co-Infection AI H9N2	Flock Age at Syndrome Occurrence Onset Time (Day)	<30								
		30-60	-1.373	0.774	0.253	0.076	-0.660	0.909	0.517	0.468
		>60	-22.050	9748.22	0.	0.998	-21.168	9420.098	0	0.998
	Abundance of Imported Chicks (Number)	<10000	-	-	-	-	-	-	-	-
		10000-20000	-0.405	0.842	0.667	0.630	-	-	-	-
		>20000	0.827	0.783	2.286	0.291	-	-	-	
	First Week Mortality (Number)	<4000	-	-	-	-	-	-	-	-
		4000-8000	1.928	0.804	6.875	0.017	1.356	0.935	3.88	0.147
		>8000	2.686	0.810	14.667	0.001	2.315	0.944	10.12	0.014
	AI H9N2+ IB+ ND Vaccine Administration	Yes	-0.405	0.551	0.667	0.462	-	-	-	-
		No	-	-	-	-	-	-	-	-
Co-Infection *ND*	Flock Age at Syndrome Occurrence Onset Time (Day)	<30	-	-	-	-	-	-	-	-
		30-60	0.454	0.515	1.574	0.378	-	-	-	-
		>60	-	-	-	-	-	-	-	-
	Abundance of Imported Chicks (Number)	<10000	-	-	-	-	-	-	-	-
		10000-20000	0.215	0.702	1.240	0.760	-	-	-	-
		>20000	0.658	0.674	1.932	0.329	-	-	-	-
	First Week Mortality (Number)	<4000	-	-	-	-	-	-	-	-
		4000-8000	-0.201	0.635	0.818	0.752	-	-	-	-
		>8000	0.154	0.624	1.167	0.805	-	-	-	-
	AI H9N2+ IB+ ND Vaccine Administration	Yes	-0.709	0.516	0.492	0.170	-	-	-	-
		No	-	-	-	-	-	-	-	-
Co-Infection *IB*	Flock Age at Syndrome Occurrence Onset Time (Day)	<30	-	-	-	-	-	-	-	-
		30-60	-0.223	1.483	0.80	0.88	-	-	-	-
		>60	-	-	-	-	-	-	-	-
	Abundance of Imported Chicks (Number)	<10000	-	-	-	-	-	-	-	-
		10000-20000	21.20	28420.72	1615474843	0.999	-	-	-	-
		>20000	0.693	1.658	2	0.676	-	-	-	-
	First Week Mortality (Number)	<4000	-	-	-	-	-	-	-	-
		4000-8000	-20.104	40192.96	0	1	-	-	-	-
		>8000	-21.869	40192.96	0	1	-	-	-	-
	AI H9N2+ IB+ ND Vaccine Administration	Yes	-21.608	17974.84	0	0.999	-	-	-	-
		No	-	-	-	-	-	-	-	-

DISCUSSION

Respiratory diseases have been one of the most important problems in broiler farming in recent years in Iran. Among these diseases, *IB*, *ND* and *AI H9N2*, which are endemic in Iran, have played the greatest role. For example according to one investigation, the sum of costs and losses due to respiratory complex was 9.47 $US Million, 2016-2017 among broiler farms in Iran. In addition to Iran, these three diseases are also the most important pathogens in the poultry farming, especially broiler one, in many other countries (Saif et al. 2020, Hosseini et al. 2015, Fallah Mehrabadi et al. 2019b, Hadipour and Golchin 2011).

In the present study, *ND* was the most frequent viral diseases in the farms contracted with multi-factorial respiratory syndrome. The disease, caused by a velogenic strain of the virus, is a significant problem in poultry farms in Iran, despite extensive vaccination efforts. The disease has been responsible for high mortality rates in recent years and continues to cause outbreaks across the country (Hosseini et al. 2014). Similar issues have been reported in other countries, such as Egypt and Pakistan, where velogenic strains of the virus have been detected in broiler chicken flocks and various bird species, respectively (Hassan et al. 2016) (Wajid et al. 2017) (Miller et al. 2015). Considering that backyard poultry are believed to be the main reservoirs of *ND*, and play a crucial role in transmitting the infection to commercial poultry farms (Awan et al. 1994), vaccinating backyard poultry could greatly reduce the reservoirs and limit the spread of the disease.

The study indicates that *AI H9N2* is a significant viral contributor to multifactorial respiratory syndrome in broiler farms across Iran, affecting over 47% of farms. The disease is endemic in Iran since 1998 which for the first time reported in the country. Although *AIV H9N2* is of low pathogenicity, its presence alongside other pathogens leads to increased mortality rates in poultry. This virus is also endemic in several neighboring countries, posing challenges to poultry farming. For instance, a study in Egypt revealed *AI H9N2* in 53% of broiler flocks with respiratory issues, with a notable co-infection rate of 41.7% alongside *IB*. Between 2000 and 2003, *AIV H9N2* was isolated from broiler chickens and quails in the UAE, where it caused mortality. In Pakistan, the virus has been endemic since 1996, resulting in ongoing economic losses (Hassan et al. 2016) (Cameron et al. 2000, Lee et al. 2016). Given the frequent mutations of *AIV H9N2* (Bashashati et al. 2013), continuous monitoring and appropriate vaccine strain selection based on the changes and the similarity between the circulating viruses are essential.

The current study found a notably low frequency of *IB* compared to the two other viral diseases examined, indicating that it had a lesser impact on the development of multi-factorial respiratory syndrome than in previous research. For example, Pourbakhsh et al in 2008 detected *IBV* in 73% of farms studied, with the 793/B genotype being the most prevalent (Pourbakhsh et al. 2008). Additionally, the Massachusetts genotype was also identified. Similarly, Seifi et al in 2010 found the virus in 40% of farms studied, with genotypes 4.91 and Massachusetts present. In neighboring countries, *IB* remains a significant issue for broiler poultry farming (Seger et al. 2016) (Hassan et al. 2016). It seems that geographic distance and control measures applied in various countries may play a role in preventing the introduction of *IBV* variants in Iran. Vaccination is crucial for the disease control, and using two different attenuated live vaccines can provide sufficient immunity against various *IBV* types as the current study supports the notion that increasing vaccination frequency can help reduce the incidence of IB.

The findings of this cross-sectional study indicate a significant relationship between flock age and the occurrence of viral diseases in broiler farms in Iran by comparing the mean age flock at syndrome occurrence time according to type of infection. Also the multivariable regression analysis further supports these observations, revealing that flocks aged at syndrome occurrence time between 30 to 60 days exhibited a higher likelihood of *AI H9N2* compared to those less than 30 days. This age-related susceptibility may be attributed to several factors, including the immunological maturity of older birds, which could influence their response to viral infections. These results underscore the importance of age as a critical factor in managing viral diseases within poultry populations. Understanding the dynamics of disease occurrence in relation to flock age can inform biosecurity measures and vaccination strategies, ultimately aiding in the control of *AI H9N2* and enhancing the overall health management practices in broiler farms. Further research is warranted to explore the underlying mechanisms of this relationship and to assess the effectiveness of interventions aimed at reducing disease prevalence in older flocks.

In this study, the role of different pathogens in various combinations in the occurrence of multi-factorial respiratory syndrome in Iranian commercial broiler chicken farms was shown. The most frequent disease was related to *ND*. In addition to *NDV*, the role of *AIV H9N2* and *IBV* in the occurrence of multi-factorial respiratory syndrome in the farms was confirmed. The occurrence of these diseases may be related to poor hygiene and lack of prophylaxis methods. Also, poor biosecurity with poor conditions of buildings and equipment may lead to exposure of poultry in farms to pathogens circulating in wild bird populations. On the other hand, due to several reasons, in addition to the change of the dominant virus, the serotypes, genotypes and circulating strains of the same virus are constantly changing. Therefore, it is crucial to prioritize viral diseases based on their prevalence and associated risk factors, determine the serotypes and genotypes of the circulating strains of the virus, and improve biosecurity measures. These strategies are essential control measures that must be implemented to mitigate the impact of multi-factorial respiratory syndrome in Iranian commercial broiler chicken farms.

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CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

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